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Bio-chemical basis in groundnut (*Arachis hypogaea*) resistant to leafminer (*Aproaerema modicella*)

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ABSTRACT

A bio-chemical study was conducted to assess the role of different plant-chemical constituents using groundnut (*Arachis hypogaea* L.) selections resistant ['ICG 5040', 'ICGS 2741', 'ICG (FDRS) 10', 'NC AC 17090' and 'GBPRS 312 (ICGV 86031)'] and susceptible ('ICG 221', 'ICGS 44' and 'Robut 33-1') to leafminer [*Aproaerema modicella* (Deventer)]. 'ICG 5040' and 'ICGS 2741' exuded more sap on injury than the other selections at 20 days after plant emergence under similar conditions. Bio-chemical analysis of complete plant-extracted sap at 50 days after plant emergence indicated that resistant selections particularly 'ICG 5040' and 'GBPRS 312' contained higher quantities of soluble sugars, nitrogen (N) and polyphenols than the susceptible ones. Analysis of phenolic compounds of the sap samples through high pressure liquid chromatography from top 2 open leaves 20 days after plant emergence indicated that the concentration of these chemicals in the resistant selections was less than in the susceptible selection. However, resistant selections were able to exude more sap, compensating for the low concentration of phenolic compounds in the sap.

Key words : groundnut, *Arachis hypogaea*, leafminer, *Aproaerema modicella*, resistance, bio-chemical, phenolics

The field and glass-house screening of groundnut (*Arachis hypogaea* L.) selections at Patancheru indicated that selections 'ICG (FDRS) 10', 'NC Ac 17090', 'ICGS 2741' and 'GBPRS 312 (ICGV 86031)' are resistant to groundnut-leafminer [*Aproaerema modicella* (Deventer)] (Amin 1987a, b, Lynch 1990, ICRISAT, Patancheru 1986, 1992) and the selection 'ICG 5040' is antibiotic to the pest (unpublished data). Visual observations on 'ICG 5040' indicated that many first-instar larvae were dying in the preliminary mines on this selection and the mine was filled up by the reddish-brown gummy exudate. Dead larvae stuck up in the gummy exudate were also observed, indicating that the exudate might have hindered the movement and caused the starvation of the affected larvae. Since sugars, nitrogen, phenolic and other compounds are known to play an important role in host-plant resistance mechanism for different insects (Miles 1969, Elliger *et al.* 1980, Mattson 1980, Chiang and Norris 1983, Hedin and Jenkins 1986, Reese 1986, Sharma and Norris 1991), we planned a bio-chemical study to identify the factors imparting resistance to groundnut against leafminer.

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MATERIALS AND METHODS

The experiments were carried out at Patancheru (18°N 78°E) during January 1992. The plants were grown in pots of 12.5 cm diameter containing soil and organic manure potting mixture. These were kept in glass-house maintained at 25–30°C and 50–70% relative humidity and were irrigated regularly. Then 200 leaflets were plucked at random from plants at 20 and 40 days and were freeze-dried. These freeze-dried samples were processed to a fine powder and used for analysis.

Total nitrogen was estimated with technicon auto analyser (Singh and Jambunathan 1980). Total soluble sugars in the sample were estimated by the method of Dubois *et al.* (1956). Polyphenols (tannic acid equivalents) were estimated by Folin Denis method (AOAC, Washington 1984). Tannic acid was used as a standard and the results were expressed as tannic acid equivalents.

The experiment on the sap material was done in 3 sets. In the first set, 8 leaflets were removed at random from each plant 20 days after emergence (20 plants/selection) and the exuding gum was collected in glass vials containing 10 ml distilled water. In the second set, sap was collected from 20 plants of each selection 50 days after plant emergence by cutting every leaflet, petiole, branch and main stem (entire plant collection) and diluted in 5 ml of distilled water. In

the third set, sap from top 2 open leaves at 20 days after plant emergence was collected into empty glass vials by removing the 8 leaflets from each selection (20 plants/selection). Since the initial sap weight were taken, the high-performance liquid chromatography (HPLC) data were interpreted as values for 1 mg sap material.

All the vials were covered with black carbon paper and stored in a cold room at 5°C. The sample materials were analysed for total soluble sugars and total nitrogen. Since the sap was highly diluted, phenolic compounds were analysed qualitatively through HPLC method after processing the sample material according to the procedure of Hahn *et al.* (1983) with slight modification in the solvent system.

Samples of 20 µl were chromatographed at a flow rate of 1 ml/min through a column RSil, C18HL (10-µ diameter particles) of 4.6 mm (internal diamter (x 250 mm (length) using a Shimadzu model LC-6A. Optimum separation was obtained by a gradient system of the following solvent mixtures: A, acetic acid-water (2 : 98) and B, butanol-methanol (8 : 92). The separation was programmed for 15 min at 20% solvent B, followed by a 50 min linear gradient to 50% solvent B. Detection was by ultraviolet absorption at 254 µm using a Shimadzu model SPD-6AV analytical detector. Retention times and peak areas (µvolts x second) were obtained with a Shimadzu CR-4A Chromatopak integrator. For qualitative estimation the detector response was calibrated for each phenolic acid.

RESULTS AND DISCUSSION

Results on soluble sugars, nitrogen and polyphenols from leaf samples (table 1) indicated that there was no distinct differences between susceptible and resistant selections for these consistents. The mean soluble sugar content of the plants at 40 days was higher by 0.51% than that at 20 days, whereas a reverse trend was observed in the levels of nitrogen and plyphenols.

The sap samples at 20 days after plant emergence had a 21.6 mg of total soluble sugars in 'GBPRS 312', whereas differences were not much in the rest of the selections that had less than half the quantity of 'GBPRS 312' (Table 2). 'GBPRS 312' and 'ICG (FDRS)' 10 had higher total nitrogen, followed by 'NC Ac 17090', whereas the rest of the selections had lower total nitrogen in their sap. Mean of soluble sugars and nitrogen was more in resistant selections than in susceptible selections at 20 days after plant emergence.

Whole-plant sap at 50 days after plant emergence had higher amount of total soluble sugars in 'ICGS 2741' followed by 'ICG 5040' and 'GBPRS 312' (Table 2). The lowest quantity of total soluble sugars was found in 'ICG (FDRS) 10', and the remaining selections had intermediate quantities. The highest quantity of total nitrogen was found in 'ICG 5040', followed by 'GBPRS 312' and 'ICG (FDRS) 10', and the lowest in 'ICGS 2741'. Except 'ICGS 2741', all the resistant selections had 2–3 times the values of phenolic compounds than the susceptible selections at 50 days after plant emergence. The highest value was observed in 'GBPRS 312' followed by 'ICG 5040'. Mean of soluble sugars, N and phenolic compounds was more in resistant selections than in susceptible selections at 50 days after plant emergence.

Thus rather than a single constituent of the plant sap, different combinations of these constituents might be responsible for contributing resistance to the groundnut selections.

The HPLC data on the phenolic compounds from the sap at 50 days after plant emergence indicated qualitative and quantitative differences in both the number of compounds, as revealed by unidentified compounds among the

Table 1 Total soluble sugars (TSS), total nitrogen and polyphenol contents in freeze-dried leaf samples of selections susceptible (S) and resistant (R) to groundnut leafminer

Selection	*S* or *R*	TSS (%)		Total N (%)		Polyphenols*	
		a	b	a	b	a	b
'Robut 33-1'	S	3.00	4.59	4.13	4.08	0.87	0.80
'ICGS 44'	S	3.35	4.04	3.96	3.80	0.99	0.78
'ICG 221'	S	3.28	3.74	3.64	3.32	1.04	0.88
Mean		3.21	4.12	3.91	3.73	0.97	0.82
'NC Ac 17090'	R	3.12	3.95	4.26	3.81	1.03	0.93
'ICGS 2741'	R	3.98	4.46	4.40	4.31	0.77	0.75
'ICG 5040'	R	2.96	3.56	3.96	4.06	0.89	0.93
'GBPRS 312'	R	3.86	3.86	3.81	4.02	0.79	0.83
'ICG (FDRS) 10'	R	3.32	3.76	4.26	4.17	0.90	0.76
Mean		3.41	3.92	4.14	4.07	0.88	0.84
SE±		0.133	0.127	0.091	0.108	0.036	0.026

a, Leaflets from potted plants at 20 days; b, leaflets from potted plants at 40 days grown in glass house at 25–30°C temperature and 50–70% relative humidity during 1992

*Tannic acid equivalents (%)

Table 2 Total soluble sugars (TSS), total nitrogen, and polyphenol contents in sap material of leaf samples of selections susceptible (S) and resistant (R) to groundnut leafminer

Selection	S or R	TSS (mg)		Total N (mg)		Polyphenols*
		a	b	a	b	b
'Robut 33-1'	S	6.1	20.1	0.03	0.13	50.2
'ICGS 44'	S	2.3	24.6	0.02	1.44	59.0
'ICG 221'	S	3.0	12.9	0.01	1.03	52.7
Mean		3.8	19.2	0.02	1.20	54.0
'NC Ac 17090'	R	7.8	22.1	0.05	1.52	106.3
'ICGS 2741'	R	2.5	67.7	0.01	0.25	7.8
'ICG 5040'	R	5.3	61.5	0.03	2.58	153.2
'GBPRS 312'	R	21.6	44.6	0.08	2.41	184.4
'ICG (FDRS) 10'	R	6.9	7.2	0.07	2.33	107.8
Mean		8.8	40.6	0.05	1.82	111.9
SE+		2.22	7.99	0.009	0.285	20.78

a, Leafletsap from potted plants at 20 days; b, whole plant sap from potted plants at 50 days grown in glass house at 25–30°C temperature and 50–70% relative humidity during 1992

*Tannic acid equivalents (%)

Table 3 Phenolics and phenolic acids of sap samples from susceptible and resistant groundnut selections

Particulars	Susceptible selection				Resistant selection					
	'Robout 33-1'	'ICGS 44'	'ICG 221'	Mean	'NC Ac 17090'	'ICGS 2741'	'ICG 5040'	'GBPRS 312'	'ICG (FDRS 10'	Mean
Sap quantity										
Fresh weight (mg)	0.1493	0.108	0.036	0.0978	0.1088	0.2394	0.522	0.1623	0.0267	0.2118
After dilution in methonol forHPLC analysis (µg/10µl)	498	360	120	326	363	1197	1740	812	89	840.2
Total polyphenols (tannic acid equilvalents %)	2.08	2.44	4.25	2.92	2.63	0.86	0.70	1.09	3.19	1.69
Phenolic acid (µg/g)										
Protocatechuic acid	1158	289	794		485	18	164	129	7753	
P-hydroxybenzoic acid	26	11	22		17	3	4	7	191	
Vanilic acid	68	14	56		3	3	7	4	90	
Coumiric acid		114					21			

Potted plants at 20 days; 8 leaflets from top 2 open leaves (20 plants/selection) were removed and the oozing sap removed and collected in dry test tubes (weighed before) and weight of sap taken later

Phendic acids were estimated by HPLC method

8 selections (Fig 1). Higher quantities of the phenolic compounds occurred at retention times of 6.3, 13.8, 14.6, 23.6 and 25.8 min for a majority of the selections.

There were 17 phenolic compounds in 'ICG 5040', the highest among the selections studied. Very little sap was extracted from 'ICG (FDRS) 10' and the further dilution resulted in no peak from the final sap processed.

Analysis of sap from the top 2 leaves at 20 days after plant emergence (Table 3) indicated that the sap quantity was highest in 'ICG 5040' followed by 'ICGS 2741' and lowest in 'ICG (FDRS) 10' and 'NCAC 17090'. It was apparent that 'ICG 5040' had exuded 19 times higher quantity of sap compared with 'ICS (FDRS) 10'. The HPLC data from this experiment are in contrast to those of the second trial (Fig 2). On an average, total polyphenols (tannic acid equivalent %) per unit quantity of sap were more in the susceptible selections when compared with the resistant ones except 'ICG (FDRS) 10' and 'NCAc 17090', indicating a negative relationship between the concentration of phenolic compounds and resistance (Table 3). Rajagopal *et al.* (1988) and Singh and Sachan (1992) made similar observations in groundnut varieties susceptible to *Spodoptera litura* Fab.

Based on the retention time, 4 phenolic acids could be identified clearly from the different HPLC peaks. Out of these, protocatechuic acid with a retention time of 6.4 min was the predominant one with the 'ICG (FDRS) 10' having

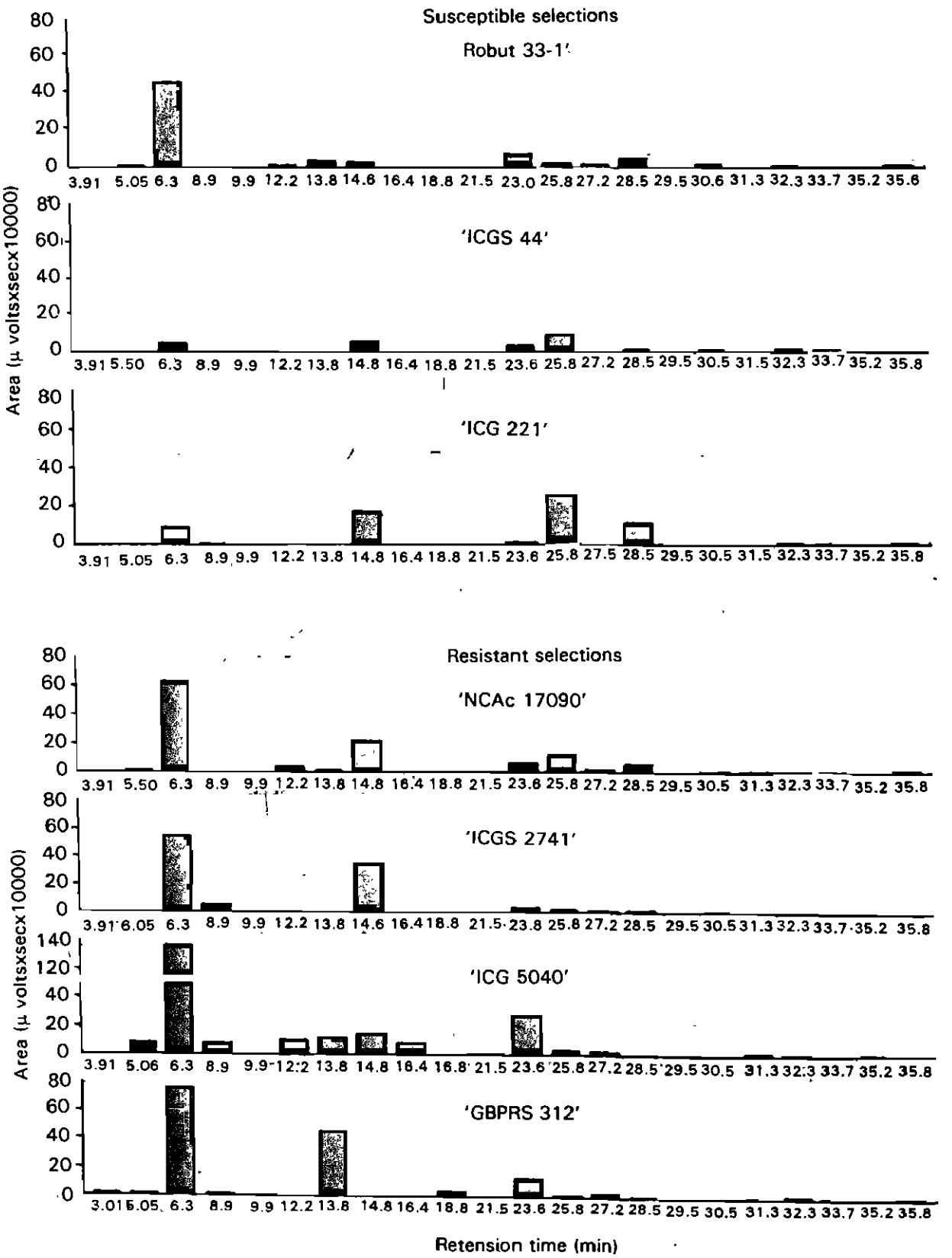

Fig 1 High performance liquid chromatographic pattern of phenolic compounds from the total sap of different groundnut selections at 50 days after plant emergence

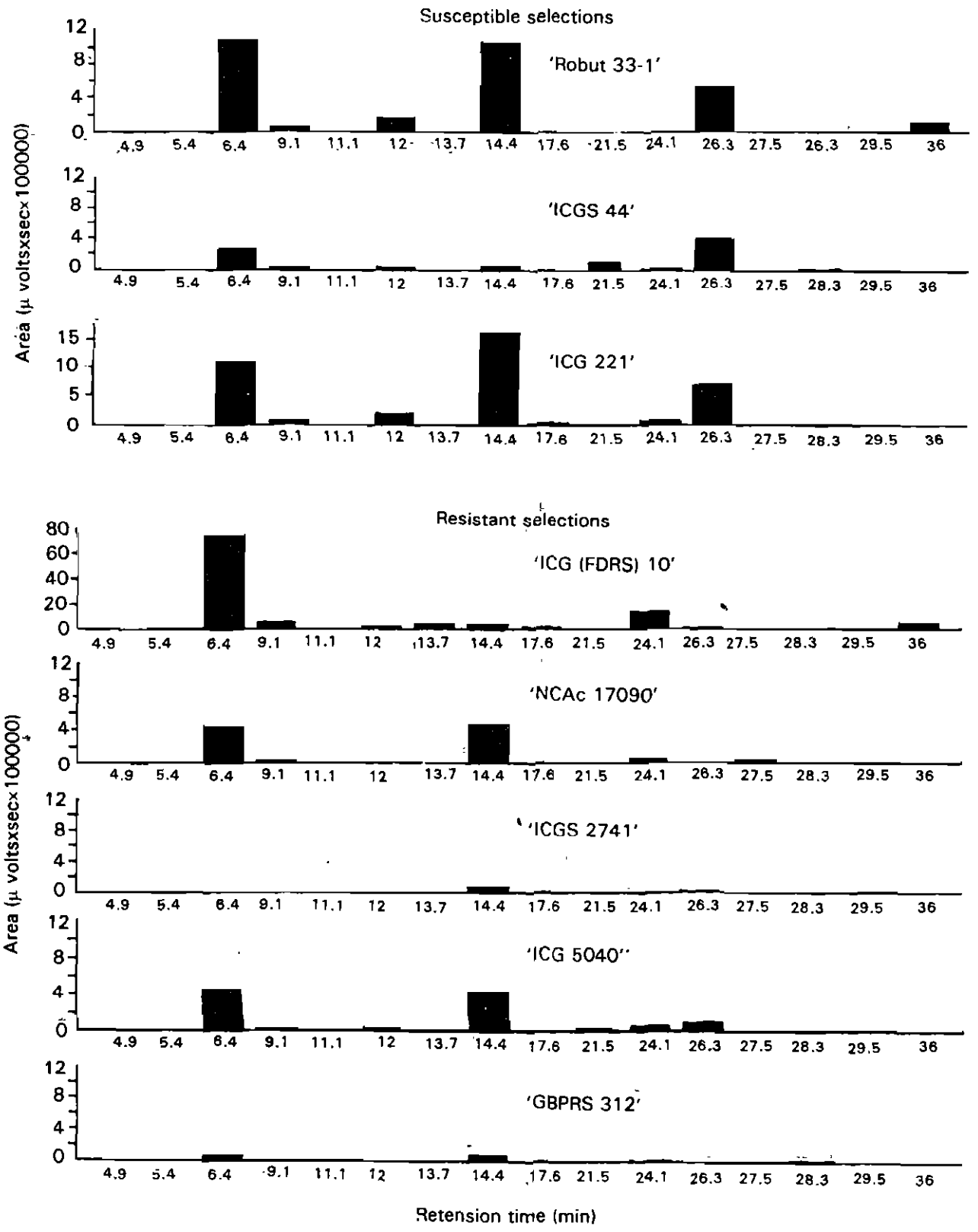

Fig 2 High performance liquid chromatographic pattern of phenolic compounds from 1 mg of sap collected from the top 2 leaves of different groundnut selections at 20 days after plant emergence

the highest concentration in the sap, followed by 'Robut 33-1' and 'ICG 221'. Selections 'ICGS 2741', 'ICG 5040' and 'GBPRS 312' had the lowest concentration of protocatechuic acid. P-hydroxybenzoic acid and vanillic acid with retention times of 9.1 and 12.0 min, respectively also followed the pattern of protocatechuic acid. Coumaric acid with a retention time of 21.5 min was present in 'ICGS 44' and 'ICG 5040' only. This also indicates that a common biochemical resistance mechanism may not be working for all the 5 resistant selections of groundnut.

In genereal, the trend indicates that in the susceptible selections the sap quantity was very low, whereas its composition of phenolic compounds washing. Though the quanity of phenolic compound was low in 'ICG 5040', grater quantity of sap high in sugars and other nitrogenous compounds was able to cause mortality of the early-instar larvae in the mine either by drowning or by taking the larvae immobile in the viscous exudate (visual observation in 'ICG 5040'). Nutritionally also the sap as well as the plant tissue might be poor in essential minor nutrients or unbalanced, resulting in higher mortality of larvae or lower pupal weight in 'ICG 5040'.

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